

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

What is claimed is:

Claims 1-42. (Cancelled)

43. (Previously Presented) A method for detecting the presence of a target nucleic acid sequence in a sample, the method comprising:

(a) adding to a sample suspected of containing the target nucleic acid sequence, a fluorescently labelled probe specific for the target nucleic acid sequence, and a DNA duplex binding agent which can absorb fluorescent energy from the fluorescent label on the probe, wherein the DNA duplex binding agent is selected from the group consisting of mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl) amino]ethyl]amino]-9,10-anthracenedione), a salt of mitoxantrone and daunomycin (8S,-cis)-8-acetyl-10[3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl]oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacendione);

(b) subjecting the thus formed mixture to an amplification reaction in which the target nucleic acid sequence is amplified;

(c) subjecting the sample to conditions under which the probe hybridises to the target nucleic acid sequence; and

(d) monitoring fluorescence from the fluorescent label on the probe,

wherein the probe is released intact from the target nucleic acid sequence.

44-45. (Cancelled)

46. (Previously Presented) The method of Claim 43 wherein the DNA binding agent is mitoxantrone.

47. (Cancelled)

48. (Previously Presented) The method of Claim 43 wherein the target nucleic acid sequence is rendered single stranded prior to hybridisation to the probe in step (c).

49. (Previously presented) The method of Claim 43 wherein the amplification reaction is the polymerase chain reaction (PCR).

50. (Previously Presented) The method of Claim 43 wherein the probe hybridises with the target nucleic acid sequence during every cycle of the amplification reaction.

51. (Previously presented) The method of Claim 50 wherein the fluorescence from the sample is monitored throughout the amplification reaction.

52. (Previously presented) The method of Claim 51 wherein fluorescence data generated is used to determine the rates of probe hybridisation.

53. (Previously Presented) The method of Claim 43 wherein the fluorescence data is used to quantitate the amount of target nucleic acid sequence present in the sample.

54. (Previously presented) The method of Claim 43 wherein the fluorescent label is a rhodamine dye, Cy5, fluorescein or a fluorescein derivative.

55. (Previously presented) The method of Claim 43 wherein the fluorescent label is attached at an end region of the probe.

56. (Previously Presented) The method of Claim 55 wherein the fluorescent label is attached at a 3' end of the probe and prevents extension thereof by a polymerase.

57. (Previously Presented) The method of Claim 43 wherein the probe is designed such that it is released intact from the target sequence during a phase of the amplification reaction other than an extension phase.

58. (Previously Presented) The method of Claim 43 wherein the probe is released intact from the target sequence during an extension phase of the amplification reaction

by action of a polymerase, and the amplification reaction is effected using a polymerase which lacks 5'-3' exonuclease activity.

59. (Previously Presented)The method of Claim 43 wherein the amplification reaction on the target nucleic acid sequence is performed in the presence of (a) a nucleic acid polymerase, (b) at least one primer capable of hybridising to the target polynucleotide, (c) an oligonucleotide probe which is capable of binding to the target polynucleotide sequence and which contains a fluorescent label and (d) the DNA duplex binding agent, and monitoring comprises monitoring changes in fluorescence during the amplification reaction.

60. (Previously presented)The method of Claim 59 wherein the amplification is suitably carried out using a pair of amplification primers.

61. (Previously presented)The method of Claim 59 wherein the nucleic acid polymerase is a thermostable polymerase.

62. (Previously Presented)The method of Claim 59, further comprising carrying out a hybridisation assay in which a hybridisation condition characteristic of the target nucleic acid sequence is measured.

63. (Previously Presented)The method of Claim 62 wherein the condition is temperature, electrochemical potential, or reaction with an enzyme or a chemical.

64. (Previously presented)The method of Claim 63 wherein the condition is temperature.

65. (Previously Presented)The method of Claim 64 wherein the method is used to detect allelic variation or a polymorphism in the target nucleic acid sequence.

66. (Previously Presented)A method for determining a characteristic of a target nucleic acid sequence, the method comprising: a) adding to a sample suspected of containing the target nucleic acid sequence, a fluorescently labelled probe specific for the target nucleic acid sequence and a DNA duplex binding agent able to absorb fluorescence from a fluorescent label on the probe, wherein the DNA duplex binding agent is selected from the group

consisting of mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl) amino]ethyl]amino]-9,10-anthracenedione), a salt of mitoxantrone and daunomycin (8S,-cis)-8-acetyl-10[3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacendione); (b) subjecting the sample to conditions under which the probe hybridises to the target nucleic acid sequence; (c) monitoring fluorescence from the sample and determining a particular reaction condition, characteristic of the target nucleic acid sequence, at which fluorescence changes as a result of the hybridisation of the probe to the target nucleic acid sequence or destabilisation of a duplex formed between the probe and the target nucleic acid sequence.

67. (Previously Presented) The method of Claim 66 wherein the reaction condition characteristic of the sequence is temperature, electrochemical potential, or reaction with an enzyme or a chemical.

68. (Previously presented) The method of Claim 67 wherein the condition is temperature.

69. (Previously presented) The method of Claim 66 wherein the results obtained from two sequences are compared in order to determine the presence of polymorphisms or variations therebetween.

70-86. (Cancelled)

87. (Previously Presented)The method of Claim 43 wherein the salt of mitoxantrone is a hydrochloride salt or a dihydrochloride salt.

88. (Previously Presented)The method of Claim 60 wherein the salt of mitoxantrone is a hydrochloride salt or a dihydrochloride salt.